Samuel Timmins, F.S.A., at Birmingham, England, on November 12, 1902, æt. 76.

Joseph Miller Wilson, at Philadelphia, on November 24, 1902, æt. 64.

Dr. Alfred Stengel read a paper on "Specific Precipitins and Their Medico-Legal Value in Distinguishing Human and Animal Blood."

SPECIFIC PRECIPITINS AND THEIR MEDICO-LEGAL VALUE IN DISTINGUISHING HUMAN AND ANIMAL BLOOD.

BY ALFRED STENGEL.

(*Read December 5, 1902.*)

Recent studies of the complex problem of immunity have been most fruitful of results, not alone in the direction of explaining immunity, but also in disclosing a number of phenomena whose bearing on the general question is perhaps subsidiary, but whose scientific and often practical interest in other directions is highly valuable. Among these the phenomenon of precipitation is an important one, and it is to this that I desire to direct attention. Kraus first showed that the serum of animals immunized against cholera causes a flocculent precipitation in the filtrate from cholera cultures, while sera from normal animals produces no such results. Later he showed that this precipitation is specific in the sense that cholera serum produces precipitation in the filtrate of cholera cultures alone, while sera from animals immunized with other cultures had no such result. Subsequent investigations have confirmed and extended Kraus' contribution. The peculiar substance which produces the precipitation in the bacterial culture is probably a product of cell activity made under the stimulus of the immunization, and the precipitable body in the cholera culture is extracted from the bodies of the bacteria themselves. It is seen then that this phenomena of precipitation as applied to bacteria is a valuable one in determining bacterial species, but it has not been so employed by bacteriologists, since it is far less easy of application and far less certain in its results than the agglutination test of Gruber, Durham and Widal.

Ehrlich's attractive theory of immunity, which he calls the Side Chain Theory, explains the development of immunity and other problems connected with this question far more satisfactorily than any previous hypothesis. He has shown very clearly that the immunizing substances are products of cell activity under the stimulus of the bacterial toxins, but he has also called attention to the fact that the principle involved in this elaboration of immunizing substances does not differ greatly from that which obtains in the case of the ordinary process of nutrition of cells. In other words, there is a chemical union of the nutrient substance with the cell body in the case of assimilation of food and a similar chemical union in the case of bacterial toxins. The molecular radicals, which have a special affinity for the nutrient body or for the toxin, are entirely comparable to the replaceable atomic groups of organic compounds, such as the benzol ring, and may be displaced from the central nucleus. Being products of the vital activity of the cell, reproduction of such atomic groups is possible, and indeed, according to a well-known principle of pathology, destruction of the atom groups occasions a replacement in excess, attended with a separation from the parent nucleus and extrusion from the cell of the atomic group. When toxins unite with cells the combining radicals or "groups" of the latter are utilized or in a sense destroyed and the cell produces new groups in excess—some or all of which are extruded into the circulation. These liberated groups constitute antitoxin in the case of immunity, and I refer to them here not from any bearing on the question of toxic immunity to our present discussion, but to develop the point that the production of such bodies is a question of chemical nature, and that it does not necessarily involve the action of a living germ. This is proved on the one hand by the production of antitoxic substances by the introduction into the organism of inorganic or organic compounds, and on the other hand by the fact that similar substances are produced when food stuffs and various other organic substances are injected. This is true in particular of the substances concerned in precipitation, and Ehrlich in his later writings has made a strong point of the similarity in the phenomena of precipitation and of bacteriolysis in so far as the origin and essence of the active agents in question are concerned.

To come more immediately to the subject, it has been found that injections of milk, albuminous liquids of other sorts and compound mixtures like urine and blood serum, when introduced into the

animal body, generate or cause the generation of substances which in a more or less specific manner precipitate the substance or compound used in the inoculations. This was first demonstrated in the case of milk by Bordet, who found that when he injected rabbits with sterilized milk and repeated the injection at short intervals the blood serum of the animals subsequently caused precipitation of the milk, while normal serum had no such action. Later it was found by other experimentors that this precipitation is more or less specific, and the serum of a rabbit treated with cow's milk contains a precipitin for cow's milk and not for the milk of other animals, while the serum of animals treated with goat's milk is similarly specific. The same experiment was performed with human milk and the result was similar. The method of carrying out these experiments was as follows: The rabbits were inoculated subcutaneously or intraperitoneally at intervals of several days with sterilized milk, the quantity varying between 10 and 50 c.c. The sterilization was accomplished by heating for an hour at 65° C. or by the use of chloroform. Other rabbits were treated with milk of different sorts as controls. After the treatment had been repeated for a number of times and each rabbit had received about 100 c.c. of milk the serum was obtained by bleeding the rabbit and allowing the blood to coagulate. The serum diluted with four or five times its bulk of water was mixed with milk diluted 1 to 40 and the mixture allowed to stand for some hours. The precipitation was then observed in the milk corresponding with that with which the animal furnishing the serum had been treated.

A very similar series of experiments has been performed with egg albumin. Repeated injections of raw egg albumin cause the development in the blood of a more or less specific precipitin. When crystallized egg albumin was used a specific precipitin was developed. This precipitates the egg albumin, but not globulin, and on the other hand, when serum globulin is used in the injections, the precipitin has no effect upon egg albumin. In the case of these more narrowly specific tests the results have been somewhat at variance. Thus it was found that the serum of animals injected with globulin obtained from bullock's serum had some effect upon blood corpuscles of fowl and also an effect upon sheep globulin. Other instances of non-specific action might be cited, but this one reference is sufficiently illustrative of all.

My own experiments in conjunction with Dr. C. Y. White in

this direction have been made with fresh egg albumin. Taking the white of fresh hen's eggs, we injected from 10 to 15 c.c. into the peritoneal cavity of rabbits. No striking effect resulted from the injections, which were repeated at intervals of a day or two until the rabbit had received from six to ten injections. At the end of that time the serum of the animal was found to precipitate diluted solutions of egg albumin in a striking manner. The same serum had no effect upon serum albumin and globulin contained in the blood of man or various animals, but was not specially tested regarding its behavior toward the albumin of other than hen's eggs. Uhlenhuth, who has experimented in this manner, found, however, that the precipitin is not specific, since the serum obtained from animals immunized with hen's eggs reacts toward pigeon's eggs. He found, however, as we did, that this serum did not react with peptone, casein, blood serum, etc.

Experiments similar to the above were made by Leclainche and Vallée, who injected albuminous urine, and Zülzer, whose work was practically the same. Mertens and Tchistovitch used eels' serum and placental blood serum respectively and found results similar in character to those already detailed.

The most interesting line of work, however, is that done by Uhlenhuth and repeated or elaborated by Wolff, Stern, Wassermann and Schütze, Dieudonne, Nuttall, as well as by myself and Dr. C. Y. White. These experiments concern themselves with the production in the blood of specific precipitins for heterologous blood, and these have been utilized to a certain extent in the determination of diseased conditions or more particularly for the differentiation of human and animal blood.

The manner of procedure in the preparation of the serum is as follows: Rabbits (which have been found to be the most suitable animals) are injected interperitoneally with blood serum or with defibrinated blood About 10 c.c. is injected at intervals of from two to three days until from five to eight or ten injections have been given. The serum may be utilized immediately after the last injection or the animal may be allowed to rest for a week before its blood is drawn. The blood is then taken after killing the animal or a small quantity may be obtained by bleeding after etherization from one of the large blood vessels. The serum is collected after separation of the clot. Fresh blood may be tested directly with the serum by diluting the blood (1 : 100) with isotonic salt solution.

The solution is made by first diluting the blood with distilled water and then with an equal quantity of double isotonic salt solution. If the diluted blood is not perfectly clear, it is allowed to settle and the supernatant blood is tested. A few drops of the serum of the immunized rabbit are added to the clear solution of blood in a narrow test-tube and a precipitation is generally observed at once or may form after standing for a short time. A later test may be performed by allowing the serum to flow down the sides of the tube under the diluted blood, when a haziness at the point of contact indicates the formation of a precipitate. The precipitate increases somewhat after its first formation, especially when the fluids are kept at 37° C., but precipitates formed twelve to twenty-four hours or more after the mixture is made should be regarded as doubtful. The same form of test may be applied with dried blood. In this case care must be taken in making solutions of the blood stains. The blood stains may be dissolved with normal salt solution, or in the case of old blood stains the method of Ziemke may be employed. In this method concentrated solution of potassium cyanide is used as a solvent. The mixture is shaken with a few granules of tartaric acid until it becomes almost neutral to litmus paper and then filtered. The mixture is diluted to a yellowish-red color and the serum of the rabbit is then added. Solutions of soda have also been used for extracting the blood clot. Uhlenhuth demonstrated the reaction in blood (1) dried for three months, (2) allowed to undergo decomposition for three months, (3) washed with weak alkaline soap, (4) frozen in snow for fourteen days at 10° C., and in (5) blood solutions in which the hemoglobin was changed to carbon oxide hemoglobin.

This test has been confirmed in more or less detail by investigators in all parts of the world and may said to be fairly established, though the limits of its usefulness cannot as yet be drawn with precision. My own experiments with Dr. C. Y. White have constituted injections of a number of rabbits with defibrinated human blood and with blood squeezed from placentæ. In both series of experiments, after from five to eight injections, we found the blood serum markedly active in precipitating diluted human blood and without effect upon the blood of dogs, horses and cattle. The first effect of adding the serum was the production of a flocculent precipitate, which invariably increased after a short time. We dis-

carded the mixtures after a few hours, as we had always observed that flocculent precipitation occurred in many of the test-tubes containing other than human blood after a lapse of twenty-four to forty-eight hours. In no case was there the slightest difficulty in distinguishing the human blood from animal blood at the primary experiment, but the greatest care was necessary in the details of the experiments to avoid any contamination. In one experiment a striking result which could not be at first explained was subsequently found to be due to a contamination.

Should this test upon further investigation prove as satisfactory as now seems probable, it will still be most essential in its practical application that the experimentor have experience, not alone with this method, but with the management of injections and serum work generally. Should the test be used in medico-legal cases, I believe that it is most essential that the experimentor's experience in serum and blood work generally be carefully scrutinized before his results are accepted.

With regard to the applicability of this test, Uhlenhuth, Stern, Nuttall, Grünbaum and others have studied the effects of the serum of the blood of various forms of monkeys and apes. Uhlenhuth had positive results with baboon's blood. Stern used three different kinds of monkeys—a species of Cercopithecus, Macacus Cynomolgus (Java Ape) and the Crown Ape. Nuttall used four species of monkeys—Cercopithecus Campbelli, Cercopithecus Patas, Cercopithecus Lalandii and Macacus Rhesus. Grünbaum has tested the gorilla, orang and chimpanzee in particular. Stern found feeble reactions with the three species of monkeys employed. Nuttall found a slight reaction with the blood of the four monkeys tested, and Grünbaum found reactions with the three animals used by him, and also found that human blood was precipitated by rapid serum from animals injected with the blood of the gorilla, orang, chimpanzee.

In a later communication Nuttall reports that in testing 140 specimens of blood none excepting monkey blood gave the reaction with the anti-serum for human blood. He states that all the bloods of the Old World monkeys gave a marked reaction, less powerful, however, than that of human blood. They also reacted to weak anti-serum. On the other hand, the South American monkey gave but a slight reaction with human dilute serum and a weak anti-serum produced no precipitation in the blood of Mycetes Senicu-

lus. He concludes that, while the tests require further study, the result corresponds with the Darwinian theory of the relationship between man and ape—the relation of the former, according to that theory, being closely to the Old World apes. The anti-serum for dog's blood when tested with 140 bloods reacted only with the blood of the jackal of South Africa. None of the 140 bloods gave a reaction with the anti-serum for horse's blood. No member of the horse family was tested.

In some cases blood serum of rabbits treated with heterologous blood have reacted in a way not specific. Thus Nuttall found the serum from a rabbit treated with ox blood reacted with the blood of the gazelle and axis deer and gave slight cloudiness with the blood of sheep, gnu, squirrel and swan. In no case, however, as far as I have discovered, has the humanized serum of rabbits reacted excepting to the blood of man or species of the monkey family.

With regard to the applicability of this test in medico-legal cases, it may be of interest to report a case studied by Prof. Wood, of Harvard Medical School. The test was applied in a murder case tried in New Hampshire. The blood of a stain on the right elbow of a brown jacket, about one-quarter inch in diameter; another, about one-half inch in diameter on the front of a jacket, were utilized. One-half of the elbow stain was cut out and soaked with distilled water and the other stain was scraped over half its extent, the scrapings being caught in a watch-glass and the powder treated with distilled water. This distilled water solution was placed in a small test-tube and treated with an equal volume of double normal salt solution. Four little spats of blood on the leg of a pair of overalls were treated in the same manner and also a stain of about one-quarter inch in diameter on the other leg. A few threads from two blood stains on a towel were cut out and a solution made as before described. Also some clotted blood found on a stone, the latter being the implement with which the murder was committed. All of these solutions were placed in small test-tubes side by side, and for purposes of comparison several other solutions of dried human blood, of that of a dog, ox, pig and sheep were employed. To each test-tube one, two or three drops of the testing serum were added. A distinct precipitation occurred within one-half hour in the test-tubes containing the blood obtained from both stains on jacket, the legs of overalls, the stain on the towel, the blood on the stone and in the test-tubes containing the human blood. No pre-

cipitation or cloudiness was obtained from the blood of the dog, pig, ox and sheep.

Several other instances of the medico-legal use of this test have been reported unofficially. The official reports will probably be published in the near future. While the reaction is not absolutely a specific one, occurring as has been pointed out in certain other animals, it seems to be reliable in distinguishing human blood from the blood of those domestic or common mammalian animals whose corpuscles most nearly resemble human blood corpuscles. While, therefore, it may not wholly replace studies of the size of corpuscles and differential counts of leucocytes to determine the percentage of different forms of granules, yet, as a method of medico-legal differentiation, it is a powerful adjuvant and is undoubtedly by far the most useful test at the present time. Some experiments in the direction of agglutination of red blood corpuscles by serum of immunized rabbits give promise that in the future this method may supplement the method of precipitation. It is too early, however, to consider this method, which has certainly not as yet reached the dignity of a practical procedure.

Stated Meeting, December 19, 1902.

President WISTAR in the Chair.

The list of donations to the Library was laid on the table and thanks were ordered for them.

The President appointed Mr. Henry Pettit to prepare an obituary notice of the late Joseph M. Wilson.

The resignations of Messrs. C. Hanford Henderson and of Samuel N. Rhoads were received and accepted.